

## Selection of Peptides Binding to HCV E2 and Inhibiting Viral Infectivity

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**The envelope glycoprotein E2 of hepatitis C virus (HCV) binds to various cell surface receptors for viral infection. We performed biopanning against this protein and selected peptides from phage display peptide libraries. Two short peptides, pep7-1 and pep12-1, were selected and their ability to inhibit the infection process was investigated. When pep7-1 was present, the infectivity of HCV particles in cell culture was notably decreased. This decrease was demonstrated by Western blot analysis, immunofluorescence assay, and reverse transcription PCR assay. However, pep12-1 showed little inhibitory effect on HCV infection.**

**Keywords:** Hepatitis C virus, E2, CD81, infection, peptide

Hepatitis C virus (HCV) causes chronic hepatitis in humans. Three percent of the worldwide population is thought to be infected [9]. More than 75% of infected patients proceed to chronic hepatitis, sometimes leading to liver cirrhosis and hepatocellular carcinoma. HCV belongs to the *Hepacivirus* genus of the Flaviviridae family. The virus contains a single-stranded positive RNA genome inside an enveloped capsid. The viral genome consists of one large open reading frame encoding a 3,000 amino-acid-long polyprotein and untranslated regions at its 5' and 3' termini. Four structural proteins and six nonstructural proteins are produced by proteolytic cleavage after translation. E1 and E2 are the two envelope glycoproteins. E2 contains a hypervariable region and a binding site for CD81, which is the major cellular receptor. Binding of E2 to CD81 commences viral attachment to host cells [5, 8] and a series of receptor interactions follows. Other receptors known to interact with HCV E2 are scavenger receptor B member I [10], tight junction proteins claudin I [3] and occludin I [1], and low-density lipoprotein receptor

[4]. Once the viral particle attaches to the cell surface, it is internalized by endocytosis in a clathrin-dependent manner [2].

JFH-1, a type 2a infectious HCV particle, has been produced in cell culture and has been widely used in research applications [7]. It is especially useful for investigating virus–cell interactions [1, 13]. There are reports describing the inhibition of viral infection using E2 antibody and sera from patients to block the interaction [6, 11]. For the same purpose, we selected short peptides binding to HCV E2 and investigated their inhibitory activities in this study.

A plasmid expressing type 2a HCV E2 was constructed. The 50-amino-acid C-terminal transmembrane domain was deleted and the rest of the protein was expressed as the 6×His-tagged form in the pQE30 vector. The protein was purified on a Ni-NTA column and was used for biopanning with a phage display peptide library (NEB, USA). One well of a 96-well plate was coated with 10 µg of purified E2 and blocked with 1% BSA. The phage solution (100 µl) was then added and allowed to bind for 2 h. Thereafter, the well was washed twenty times with a TBS buffer, and bound phages were eluted by lowering the pH to 2.2. The eluted phages were pooled and amplified by infecting ER2738 cells. These amplified phages were then used for a new round of biopanning, and the process was repeated 3 to 5 times until certain peptide sequences were shown to be enriched. Phages were collected and their DNA was isolated for nucleotide sequence analysis (Solgent, Korea). Pep7-1 and pep7-2 showed similar deduced amino acid sequences with a region consisting of 4 residues (Table 1). Peps 12-1 through 12-3 showed similar sequences with a conserved block of 8 amino acids. Pep7-1 and pep12-1 were selected for further investigation of their inhibitory activities against viral infection. The two peptides were synthesized with N-terminal acetylation and C-terminal amidation (Pepton, Korea). HCV particles were obtained by electroporation of *in vitro* transcribed JFH-1 RNA into Huh-7.5 cells (kindly provided by C. Rice) [13]. Culture supernatant was obtained 10 days post transfection and

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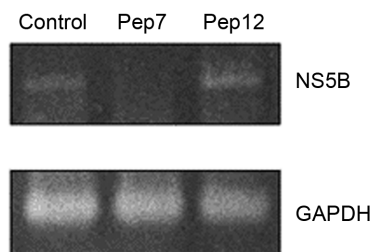
**Table 1.** List of selected peptides binding to HCV E2.

Peptide name	Amino acid sequences
7-1	<u>TSQNIRS</u>
7-2	<u>TSYHRSA</u>
12-1	<u>SVSVGMKPSRP</u>
12-2	<u>SVSVGTKPRRP</u>
12-3	<u>SVSWGMPSPRQ</u>
Control	STLPLPP

Conserved amino acids are underlined.

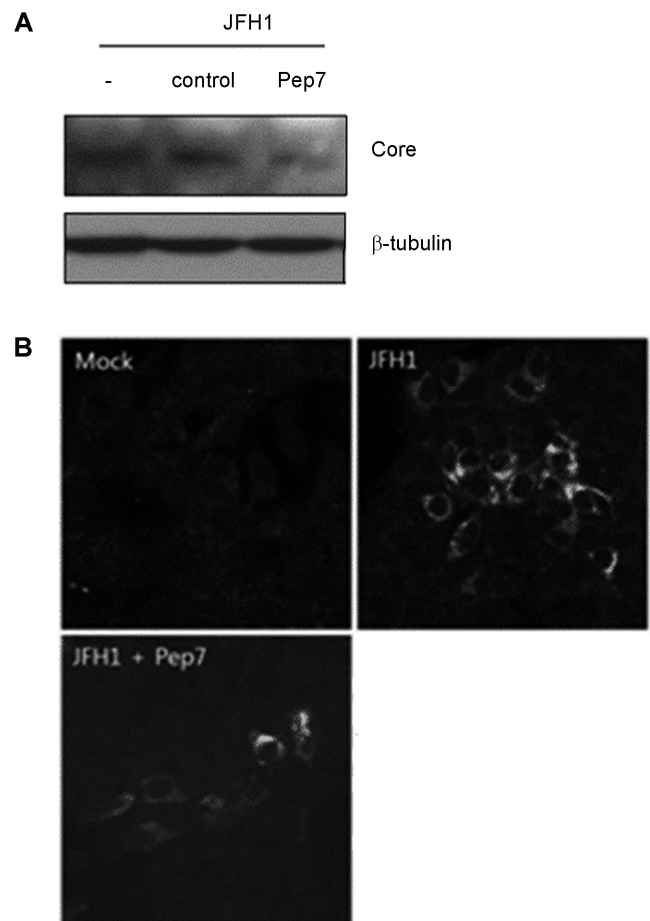
was used for the *in vitro* infection assay. The culture supernatant was incubated with or without each synthetic peptide at a concentration of 60  $\mu$ M at room temperature for 1 h before adding to fresh Huh-7.5 cells. The cells were washed three times in 6 h and further incubated for 48 h. Total RNA was isolated and RT-PCR was performed with primers specific for the NS5B region. In the presence of pep7-1, the HCV RNA level was greatly reduced compared with that in the presence of control peptide (Fig. 1). On the other hand, pep12-1 did not affect the HCV RNA level. The inhibitory effect of pep7-1 on HCV infection was confirmed using anti-core antiserum raised against the 2a strain (a kind gift from S. B. Hwang). Cell lysates were obtained 48 h after infection with supernatant containing JFH-1 particles, and Western blotting was carried out with anti-core antibody (Fig. 2A). In the presence of pep7-1, the reduction in HCV core protein was prominent. Immunofluorescence confocal microscopy was used to verify the results. Cells were infected with supernatant containing infectious JFH-1 particles in the presence or absence of pep7-1. In the presence of pep7-1, only a fraction of the cells were stained with anti-core antibody compared with the control (Fig. 2B).

We successfully isolated peptides binding to HCV E2 using biopanning with a phage display peptide library. Pep7-1 showed inhibitory effects against HCV infection in

**Fig. 1.** Replication of viral RNA in the presence of pep7-1 or pep12-1.

RT-PCR of the HCV NS5B region (plus strand) was performed using JFH-1-infected cell lysates in the presence of control peptide, pep7-1, or pep12-1. Total RNA was obtained with RNeasy Mini Kit (Qiagen, Germany) and RT-PCR was performed with appropriate primers. GAPDH was used as an internal control.

cell culture. This peptide is thought to inhibit binding of the virus to the receptor for two reasons. First, the peptide was isolated using a biopanning process, yielding highly specific interaction partners. Random binders are eliminated during repeated panning processes. The appearance of conserved amino acid regions at the end of the process supports the specific interaction between pep7-1 and HCV E2. Second, if we assume that the peptide cannot inhibit binding between E2 and the receptor, it should be internalized along with the particle to produce the inhibitory effects. Once inside the cell, however, the peptide is usually easily degraded and cannot reach the endoplasmic reticulum where viral replication (*e.g.*, RNA polymerization, proteolytic cleavage, and viral assembly) takes place. It should remain with E2 attached to the plasma membrane. Moreover,

**Fig. 2.** Detection of HCV core protein after infection of Huh-7.5 cells with JFH-1 particles in the presence of pep7-1.

A. Western blot analysis was performed 48 h post infection in the absence of added peptide, in the presence of control peptide, or in the presence of pep7-1. Tubulin was used as an internal control. B. Immunofluorescence confocal microscopy analysis of HCV core, produced 48 h post infection in the presence of control peptide (JFH1) or in the presence of pep7-1 (JFH1 + pep7). Mock-infected control is shown to illustrate the specificity of the anti-core antibody.

considering the number of peptide molecules needed for inhibition of viral replication, it is highly unlikely that only a few molecules of peptide internalized with E2 could inhibit a viral replication process mediated by hundreds of copies of viral proteins. Thus, it seems that pep7-1 inhibited the binding of E2 to cellular receptors.

Although we also isolated pep12-1 as a highly specific interacting partner for HCV E2, it showed little inhibitory effect. As compared with pep7-1, which consists of 6 hydrophilic amino acids out of 7, pep12-1 consists of 5 hydrophilic amino acids out of 12. The lower solubility of the latter peptide could be one reason for its lack of inhibitory activity. Another possibility is the location of the binding site. A direct masking of the receptor interaction site or allosteric modulation is needed for inhibitory activity. Pep12-1 may lack both.

In the case of HIV, a long list of developments in entry inhibitors has been reported [15]. As the process of viral entry into host cells is increasingly revealed for HCV, the current study can provide a clue regarding its entry mechanism and the development of a candidate lead molecule.

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